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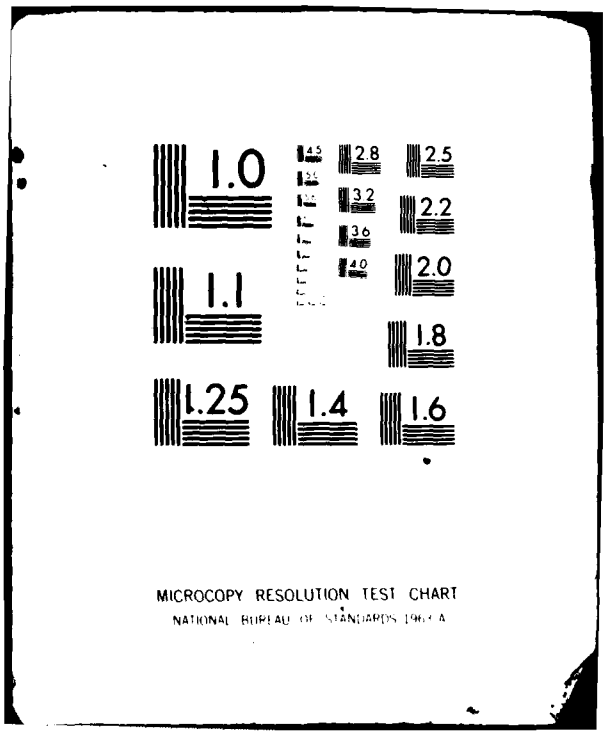
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Although present laser safety standards are based on an adequate data base for acute viewing situations, they are limited in predicting the type of change in visual function that might be induced from prolonged or repetitive viewing of laser sources. Viewing requirements in holography, laser display systems, and, in general, repeated exposure to low levels of laser radiation require a more complete data base for optimizing the environmental protection of individuals who will be required to work in such environments. In these studies, we have simulated very low-level radiation environments and determined the effects of

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repetitive prolonged exposure on the visual function of the Rhesus. Our data suggest that prolonged viewing of such sources, even though they are well below present laser safety standards, can produce permanent changes in visual processes that underline normal human day (photopic) and night (scotopic) vision. Studies of morphology have shown possible subtle morphological correlate. The coherency of laser light is implicated as a significant factor in inducing these effects. It is recommended that individuals required to work in these situations be frequently evaluated for changes in visual function by presently available clinical instruments for assessment of visual function. Further confirmation of these studies will determine the impact of these research findings on present laser safety standards.

TECHNICAL NOTE NO. 81–19TN

LONG-TERM AND PROGRESSIVE CHANGES IN RHESUS SPECTRAL SENSITIVITY AFTER LOW-LEVEL LIGHT (514 nm) EXPOSURE

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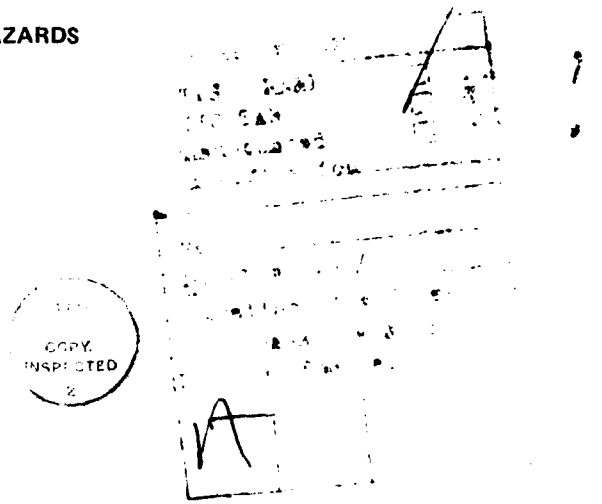

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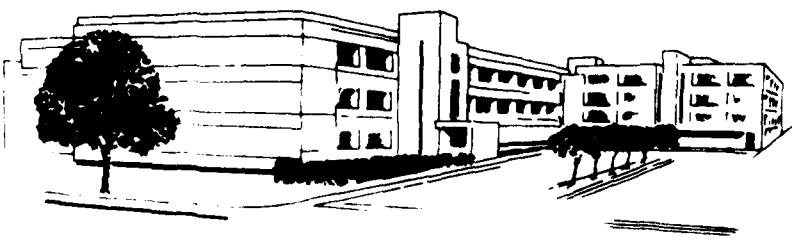

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Long-term and Progressive Changes in Rhesus Spectral Sensitivity after Low-level Light (514 nm) Exposure--Zwick et al

In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

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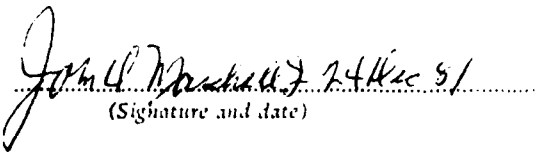

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LONG-TERM AND PROGRESSIVE CHANGES IN RHESUS SPECTRAL SENSITIVITY AFTER LOW-LEVEL COHERENT LIGHT (514 nm) EXPOSURE

In a previous investigation (1), we showed that low-level exposure to repetitive, diffuse argon laser radiation can permanently depress intermediate spectral sensitivity and produce an effect similar to that reported by Harwerth and Sperling (2) for intermediate incoherent spectral light. However, unlike their effects (2), our sensitivity functions failed to show recovery. We've used retinal irradiance levels about 1000 times lower than those used by these investigators (2). This investigation presents additional data for one animal more than two years after exposure, and evidence of gradual decline in sensitivity long after cessation of exposure in the case of two animals. Electroretinographic (ERG) spectral sensitivity measurements indicate that these effects originate at the retina.

METHOD

A detailed description of the apparatus and training procedure has been provided previously (3,4). Spectral sensitivity functions were determined in each session by determining the log threshold background intensity required for discrimination of a specific acuity criterion at each wavelength. These determinations were made by an up-and-down threshold procedure in which Landolt and gapless rings were presented sequentially in sets of four rings of equal diameter. Three rings were gapless and the position of the fourth ring, a Landolt, ring was always randomized within the set. Correct responses by the animals to Landolt rings decreased background intensity by 0.2 log units, whereas incorrect responses to Landolt rings increased background intensity by 0.2 log units.

Measurements of threshold intensity were made every 20 nm through the visible spectrum in a quasi-random order. All

measurements of background intensity were normalized for quantal flux at 600 nm. Spectral sensitivity was measured for various Landolt ring gap sizes from our largest or coarsest gap at 0.14 min^{-1}, to our smallest or finest at 1.85 min^{-1} (1.85 min^{-1} = 0.5 min of arc).

Behavioral data from two mature rhesus monkeys were obtained. Animals were emmetropic (no optical correction was required). Funduscopic examination of both eyes before and after exposure revealed no evidence of light-induced funduscopic change. Each animal was chaired and enclosed in a standard primate chamber that attenuated extraneous noise and light. A plexiglass head restraint minimized the ability of the animal to move his head during experimental sessions. The beam from an argon laser (Spectra Physics Model No. 164) was reflected into the primate cubicle from behind the animal's head and was diffused by a small (5 x 5 cm) ground glass slide located outside the direct view of the animal (Fig 1). Forward scatter from this diffuser almost uniformly irradiated a hemisphere

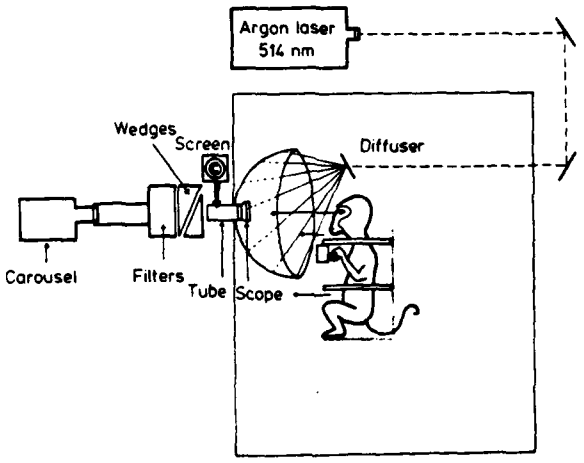

Figure 1. Schematic drawing of rhesus in experimental apparatus. The beam from an Ar laser (514 nm) was diffused at a small diffusing plate behind the animal's head and outside of its view. Blocking plates (not shown) were routinely used to fix or restrict animal's head movements. The radiance of the hemisphere was 6·1 $\mu W\,cm^{-2}\,sr^{-1}$. Assuming a 3 mm pupil during exposure, this radiance corresponds to an average retinal illuminance of 2·25 log *td and an average* retinal irradiance of 0·2 $\mu W\,cm^{-2}$ over the entire retina.

painted flat white, (radius, 0.5 meters). The location of the animal's head was approximately at the center of the hemisphere. The animal viewed the rear projected stimuli and background through a 5 cm diameter tube protruding 6 cm into the hemisphere. Viewing of the stimuli was binocular. The average of the measured luminance of the hemisphere was $\pm$25 nits. Radiometrically, the average irradiance of the hemisphere was approxmately 20 uW cm^{-2} over the entire retina (assuming a 3 mm pupil). Retinal illuminance was 2.25 log td. The radiance of the hemisphere was 6 uW cm^{-2} sr^{-1}.

Retinal spectral sensitivity measurements were made for a low-level ERG criteria (0.5 uV_{rms}), by using a lock-in amplifier technique. Exposure to 514 nm was made in Maxwellian view for a

visual angle of 55 degrees. Test stimuli subtended 4.2 degrees. The retinal irradiance equaled 12.5 uW cm^{-2}. Data from this animal were compared with that obtained from behavioral animals so that the extent of scotopic intrusion on both of these functional criteria could be assessed (Figure 6).

RESULTS

Changes in log relative sensitivity for two animals at a single monochromatic background (520 nm) are shown in Fig 2. Arrows on the abscissa indicate exposure days. Each exposure was 2 h for a total

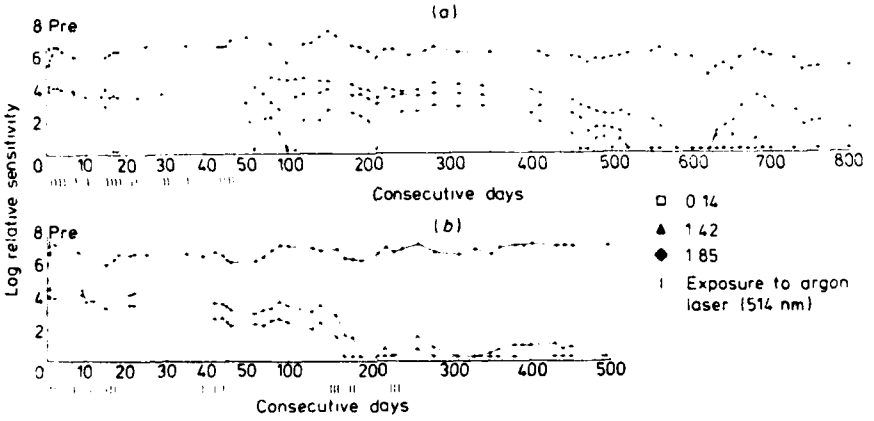

Figure 2. Log relative sensitivity at 520 nm as a function of consecutive days, including exposure period and post-exposure period for two subjects. Each vertical arrow on the abscissa represents a 2 h exposure session. Each animal received a total dose of 38 h, although the density of exposure differed. Both animals showed earliest loss in sensitivity for the highest acuity criterion, 1·85 min^{-1}. In both cases, loss at high photopic acuity criteria was permanent, although more 'waxing and waning' are evident for our first animal (*a*) than for the second (*b*). Both animals showed much less change in sensitivity for the coarsest acuity criterion, 0·14 min^{-1}, than for any of the higher acuity criteria.

of 38 h given each animal. The exposure given to our first animal is shown in Fig 2(a). Sensitivity for four acuity criteria (1.85, 1.42, 0.98, and 0.14 min^{-1}) has been tracked for more than 2 1/2 years. The first sign of change in this measurement became apparent after 10 h exposure at the 1.85 min^{-1} criterion, our finest gap size. By 18 h total exposure, measurements at this criterion were no longer obtainable with our apparatus. Measurements of the entire dark-adapted spectral sensitivity were also not obtainable from this time to about 3 months after exposure (Fig 3).

Sensitivity for 520 nm at coarser but still photopic criteria (1.42 and 0.98 min^{-1}) showed relatively little change throughout the total exposure period. Two weeks after the last exposure, however, sensitivity at the 1.42 min^{-1} criterion declined sharply and was not

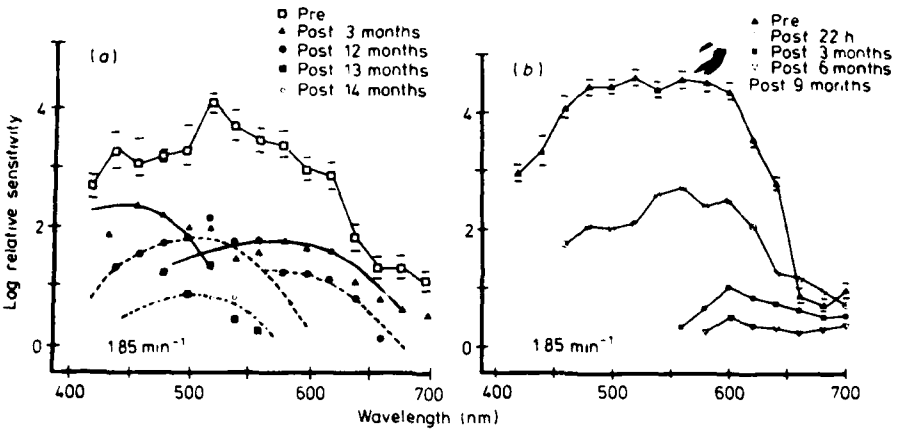

Figure 3. Pre- and post-exposure dark-adapted spectral sensitivity functions at 1·85 min^{-1} are shown for both animals. Post-exposure spectral sensitivity functions for the first animal (*a*) are shown at 3, 12, 13 and 14 months. Data obtained at 3 months post-exposure were capable of being fitted with the short-wavelength photopigment nomogram. However, in subsequent measurements the short-wavelength nomogram was replaced by the CIE scotopic function to better fit data in the short and intermediate spectral regions. After 14 months, no measurements at this criterion were possible. (*b*) Measurements of spectral sensitivity at the 1·85 min^{-1} acuity criterion obtained before, during, and after cessation of exposure for the second animal. Sensitivity for this animal also declined after exposure ceased and measurements were no longer possible after 8–9 months post-exposure.

obtainable for several weeks. Measurements made at the 0.98 min^{-1} acuity criterion were increased in frequency, and also reflected a decline in sensitivity by almost a log unit. Several weeks later, sensitivity at the 1.42 min^{-1} and the 1.85 min^{-1} acuity criteria returned.

Long-term measurements, continued for more than 2 1/2 years after the last exposure, have shown a gradual decline in sensitivity at 1.85 and 1.42 min^{-1} acuity criteria. At present, measurements of sensitivity at 520 nm for these acuity criteria are unobtainable. Increased "waxing and waning" are evident for the 0.98 min^{-1} criterion. Much less change in sensitivity has occurred at our coarsest acuity criterion (0.14 min^{-1}) over this same time period.

Our second animal received 22 h (Figure 2(b)) total exposure over approximately the same time frame (40-45 days) in which our first animal received its total cumulative exposure of 38 h. The earliest changes in this animal occurred between 18 and 22 h total exposure. A gradual decline in sensitivity was evident after 22 h exposure for our highest acuity criterion. Ten more exposures virtually eliminated our capacity to measure sensitivity at 520 nm for the 1.85 and 1.42 min^{-1} criteria. Drop off in sensitivity was much more rapid for this animal and showed much less evidence of

"waxing and waning" in sensitivity than our first animal exhibited over a comparable time frame. At the end of about 500 days, sensitivity at 1.85 and 1.42 min^{-1} was either not measurable, or barely so with our apparatus. Sensitivity at 0.14 min^{-1}, our coarsest criterion gap, remained fairly unchanged during this time, although evidence of a slight increase in sensitivity is present.

In Figure 3(a), long-term post-exposure changes in spectral sensitivity for our finest criterion, 1.85 min^{-1}, are shown for the two animals presented in Figure 2. For our first animal, spectral sensitivity at this criterion was no longer measurable after 18 h total exposure (Figure 2(a)). Spectral sensitivity measurements were not possible until approximately three months after exposure. At this time, it was possible to fit a long-wavelength cone nomogram and a short-wavelength cone nomogram. The maximum loss in sensitivity appeared in the intermediate spectral region. However, the fit in the short end of the spectrum became increasingly more difficult to make with continued measurement. At 12 months after exposure, spectral sensitivity had decreased and shifted in peak wavelength so that the Commission Internationale de l'Eclairage scotopic function now made a more reasonable fit to the short-wavelength region. Long-wavelength sensitivity decreased at this criterion, and measurement of the entire spectral sensitivity curve was unobtainable 14-16 months after exposure.

Figure 3(b) corresponds to the spectral sensitivity of our second animal (Fig 2(b)), also measured for our finest acuity criterion and tracked over a 9-month period. After 22 h total exposure, spectral sensitivity at this criterion dropped nearly two log units across the spectrum and showed a slight peak shift in maximal sensitivity toward the longer wavelengths. At this time, this animal had received a total exposure of 22 h over approximately the same period of days that our first animal received its total 38 h exposure. Decline in sensitivity from three months to six months is evident. At 9 months, measurements were no longer possible at this acuity criterion.

Figure 4 shows changes in spectral sensitivity at the 1.42 and 0.98 min^{-1} criteria for our first animal. Decline in sensitivity is slower for these criteria than for the 1.85 min^{-1} criterion. Nevertheless, at 1.42 min^{-1} measurements were no longer possible 20 months after exposure, while much reduced sensitivity was obtainable at 30 months after exposure for 0.98 min^{-1}. Furthermore, sensitivity at both criteria underwent broadening in the long-wavelength spectral region, and shifted in peak spectral sensitivity toward the long-wavelengths for measurements made more than a year after exposure.

At the coarsest acuity criterion employed (0.14 min^{-1}), sensitivity showed little decline over 18 months after exposure (Figure 5). Presently, loss in the short and intermediate spectrum

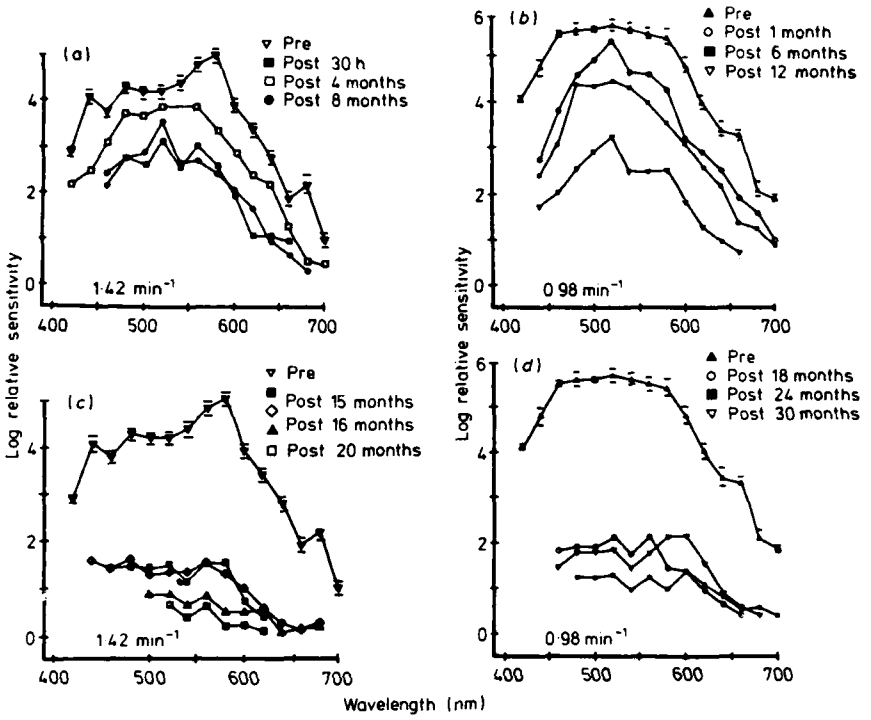

Figure 4. At 1·42 and 0·98 min^{-1} spectral sensitivity declined more slowly than that at 1·85 min^{-1}, as evidenced by these data from our first animal. At 1·42 min^{-1}, spectral sensitivity measurements were no longer possible 20 months post-exposure, while more gradual decline in sensitivity was noted for the 0·98 min^{-1} acuity criterion. In both these criteria, spectral sensitivity reduction is accompanied by an apparent broadening of the functions in the long end of the spectrum.

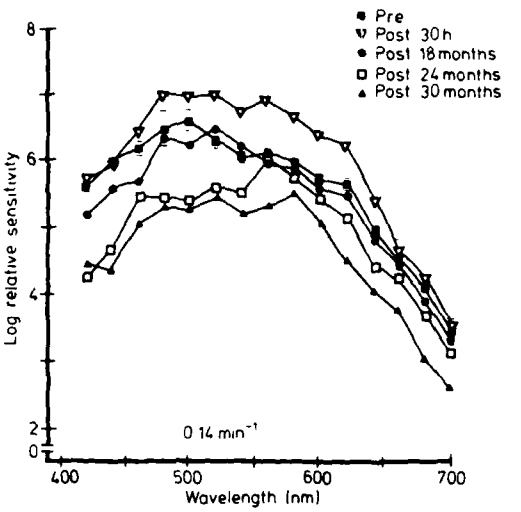

Figure 5. Spectral sensitivity at $0{\cdot}14\ min^{-1}$ for our first animal showed relatively little decline in sensitivity over an 18–24 month post-exposure period. Some loss in the short and intermediate spectral region became evident after 24 months post-exposure. Also note that at the end of 30 h total exposure, spectral sensitivity at this criterion was increased by about one log unit across the spectrum. In our second animal, measurements at this criterion have so far been made over 12 months post-exposure with very little loss in overall sensitivity, and as yet no apparent shift in peak wavelength.

has become evident with little change in the long-wavelength region. A peak in the long-wavelength region may be developing at this criterion. Changes in spectral sensitivity at this acuity criterion for our second animal have been minimal, and similar to those obtained with our initial subject. In both of these animals, as well as several others of similar age, baseline measurements (before exposure) of spectral sensitivity made over 1 to 2 years showed little change. Changes that did occur were non specific to acuity criteria, and were never wavelength-specific in the manner we have described.

In Figure 6(a), measurements at 1.42 min^{-1} taken after 10 h of chromatic exposure are compared with measurements taken at 30 h total exposure, and both curves are normalized with the Commission Internationale de l'Eclairage (CIE) scotopic curve at 520 nm. The behavioral spectral sensitivity measurements made at 30 h of exposure indicate closer agreement with the Commission Internationale de l'Eclairage (CIE) scotopic curve than the behavioral function measured at 10 h. A similar correspondence is observed for measurements made with ERG criteria, (Fig 6(b)). Data from one rhesus monkey are shown before and after a single 2 h exposure. Post-exposure spectral sensitivity measurements made 2 months later conform better than pre-exposure measurements to the Commission Internationale de l'Eclairage (CIE) scotopic curve. Changes in absolute spectral sensitivity for ERG criteria also paralleled the behavioral data. At 40 Hz, where most of the neural component was cone dominated, absolute depression in sensitivity of at least a log unit was obtained, while enhanced sensitivity was observed at lower chopping frequencies where rods dominated the lock-in ERG criteria.

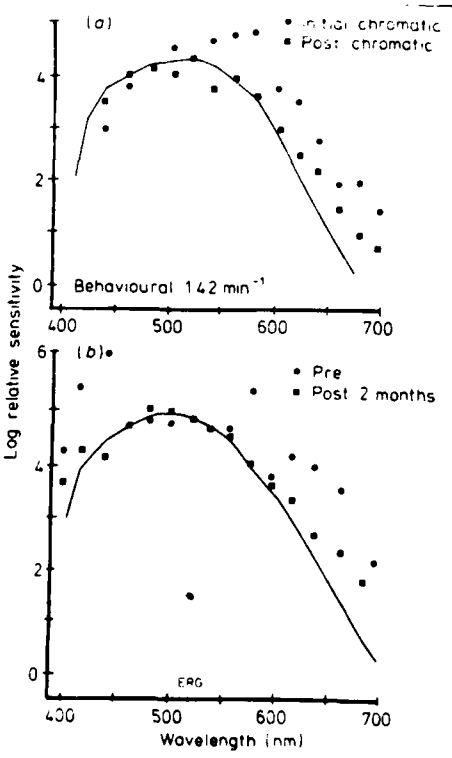

Figure 6. A comparison of exposure effects on behavioural and ERG spectral sensitivity. In (*a*), behaviourally determined spectral sensitivity measured after 10 and 30h total exposure have been normalised to the CIE scotopic function at 520 nm. The 30h function makes a better fit with the scotopic function than the 10 h function. In (*b*), pre- and post-exposure ERG spectral sensitivity functions are compared in a similar manner, and also indicate that exposure produces better conformity with the scotopic function.

DISCUSSION

The data presented here indicate that low-level, prolonged, repetitive viewing of visible laser light at 514 nm can substantially depress photopic function, and that such effects have retinal origin as evidenced by our ERG studies. Long-term follow-up measurements indicate that such photopic depression is most severe for our highest acuity criteria, which suggests that foveolar visual processes are permanently altered, although the full effect may be delayed for many months after the cessation of exposure. Similar observations at our lower, but still photopic, acuity criteria indicate that the decline in spectral sensitivity at these criteria is slower but still progressive. Little change in sensitivity at our coarsest acuity criterion was observed out to 18 months after exposure. At 24 months, the significant peak shift which had occurred is suggestive of loss of photoreceptor function in the short and intermedate spectral regions and may reflect changes in rod function and/or changes in more peripheral central cone processes absorbing in these wavelength regions. In other investigations, our studies (5) indicate that spectral sensitivity for this acuity criterion can involve both rod and cone function.

Further comparison of our two animals suggests a possible cumulative process. Our first animal received 38 h total exposure over a 45-day period. Measurements of spectral sensitivity at 1.85 min^{-1} were not possible up to 3 months after termination of exposure. Our second animal received a total exposure of 22 h over approximately the same period of days, and had a measurable but altered spectral sensitivity at this criterion acuity. This effect showed no recovery between the 22nd hour of exposure and the next exposure session. In fact, a gradual decline in sensitivity occurred, which suggests the same process in this animal was initiated, but in milder form.

In Figure 7, we have compared the retinal irradiance levels used in our experiments with those used by other investigators.

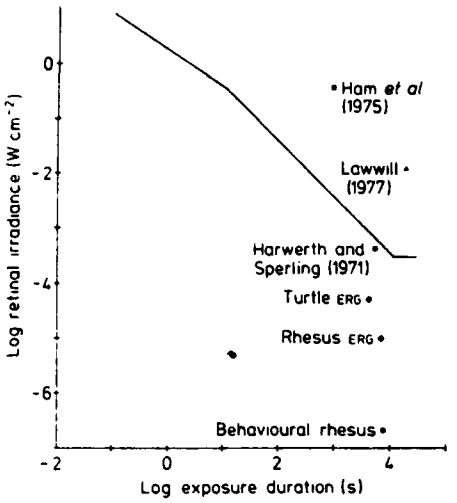

Figure 7. The full line shows the calculated permissible retinal irradiance for extended source laser viewing, and relevant data from other rhesus investigations where changes in morphology and function were induced from intermediate spectral exposure (514, 520 nm). The level used by Harwerth and Sperling (1971) was about 1000 times higher than that used in our investigation for filtered white light at a peak wavelength of 520 nm. Other investigations where laser sources were used at 514 nm (single exposure and morphological criteria) are shown (Ham *et al* 1975, Lawwill *et al* 1977) in which morphological changes were obtained with at least 10 times above the calculated permissable retinal irradiance. The data of Ham *et al* represent a threshold determination of the energy required to produce funduscopic alteration. The retinal irradiance of Lawwill *et al* represents a level that produced morphological alteration throughout the various layers of the retina. Levels which produced change in our ERG and behavioural measurements are also designated. (Turtle ERG refers to an irradiance level from Zwick and Jenkins (1979) obtained at 620 nm.)

Because the exposure levels in our experiments are much lower than those used by Harwerth and Sperling (2), and the spectral sensitivity early as 18 h after exposure, it is quite possible that our effects are unique to the characteristics of the laser source, as well as to the peak wavelength of the stimulating source (2). The coherency of a laser source produces a unique interference phenomenon when interactive with a surface. Such speckle patterns may pose unique stimulus characteristics to the retina and the visual process itself. Laser speckle is highly monochromatic, compressing light quanta at 514 nm into a spectral bandwidth of less than 0.05 nm. Spatial frequency measurements, made in our animal's hemisphere, of the laser speckle from the diffuse 514 nm source indicate the presence of fine spatial frequencies that may be reproduced at the retina (9). Although such spatial frequencies are rare, they can occur with peak irradiances much greater than those given by our average irradiance. Such small diffraction-limited spots from lasers are capable of being imaged on the retina, as laser speckle does not seem to be affected by the eye's optical apparatus (10). Unique saturation of photoreceptors may result, therefore, from an array of such stimuli moving over the entire retina. In our succeeding experiments, we have demonstrated that greatly attenuating this speckle pattern will significantly alter the obtained effects on visual processes in turtles (8,11). Other studies (12-14) indicate

that cone photoreceptors are more susceptible to alteration than are rods, which suggests, that the uniqueness of the laser source may be related to characteristics of the cone photoreceptors that are not possessed by rod photoreceptors.

Finally, the nature of the changes observed in these experiments remains to be elucidated. Although we presently have no morphological parallel to this study, the progressive decline in cone spectral sensitivity would indicate long-term suppression of the neural output of these receptors. Whether or not such effects are permanent and indicative of an injury process may require morphological correlation. Whether or not our effects are ultimately recoverable with time or through alternative stimulation is also unknown. It seems plausible to us at the present time to entertain two hypotheses: first, that we are dealing with a progressive central retinal injury process precipitated by prolonged laser light exposure; or, secondly, that we are dealing with a long-term adaptive process that has been evoked by a unique input to the visual system and paradoxically could be protective in function.

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